

## **REMARKS**

Entry of this Amendment is proper under 37 C.F.R. § 1.116 because the Amendment places the application in condition for allowance for the reasons discussed herein; does not raise any new issue requiring further search and/or consideration because the amendments amplify issues previously discussed throughout prosecution; relates to matters of form rather than substance because the added language was already present in the claims, and places the application in better form for an appeal should an appeal be necessary. The Amendment is necessary and was not earlier presented because it is made in response to arguments raised in the final rejection. Entry of the Amendment, reexamination and further and favorable reconsideration of the subject application in light of the following remarks, pursuant to and consistent with 37 C.F.R. § 1.116, are thus respectfully requested.

### **1. Status of the Claims**

Claims 1-6, 8-11, and 20-35 stand pending. Claims 33-35 stand withdrawn. Claims 1-6, 8-11, and 20-32 stand rejected.

After entry of the above amendments, claims 6-24 and 28-35 stand cancelled, including claims 33-35, which had been withdrawn as drawn to a separate invention.

Applicants have not amended any of the claims but instead introduce new claims 36-39. The new claims are supported by at least the original claims as well as Examples 2 and 4 and Tables 2 and 3. Applicants do not believe that the amendments add subject matter that is unsupported in the Specification as filed. Accordingly, no prohibited new matter is introduced by the entry of the amendments.

Claims 6-24 and 28-35 have been cancelled without prejudice to, or disclaimer of, the cancelled subject matter. Applicants reserve the right to file a continuation or divisional application on any subject matter canceled by way of amendments.

### **2. Information Disclosure Statement**

Applicants note with appreciation the acknowledgement of the Information Disclosure Statement filed February 8, 2008.

**3. Withdrawal of the Claims**

The Office withdraws claims 33-35 from consideration as being directed to a non-elected invention. Specifically, the Office alleges that the previously submitted claims 33-35 are distinct species from the originally claims for being directed at pharmaceutical compositions “comprising an *SDS*-rich product.” Page 2, Office Action (emphasis added). The present application only describes secoisolariciresinol diglycoside (*SDG*); therefore, Applicants assume the Office means “an *SDG*-rich product.”

Applicants submit that the Office has not adduced a proper basis for why claims 33-35 cannot be examined with the present invention. No explanation as to separate class of invention is set forth and no explanation of burden. Additionally, the claims depend from claims presently under examination and are thus linked.

Nevertheless, in order to expedite examination, Applicants have canceled claims 33-35.

**4. Rejection under 35 U.S.C. § 112, First Paragraph (Written Description)**

The Office rejects claims 1-5 and 25-27 under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. The Office alleges that claims 1-5 and 25-27 contain subject matter that was not described in the Specification in such a way as to reasonably convey to one skilled in the relevant art that Applicants had possession of the claimed invention at the time the application was filed.

Applicants traverse the rejection. As long as enough detail in the specification has been set forth to allow an ordinarily skilled in the art (1) to understand what is claimed and (2) to recognize that the named inventor(s) invented what is claimed, the written description of 35 U.S.C. § 112, first paragraph, has been satisfied. *Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 921, 69 U.S.P.Q.2d 1886, 1896 (Fed. Cir. 2004). Moreover, “a patent applicant does not need to include in the specification that which is already known to and available to one of ordinary skill in the art.” *Koito Mfg. Co. v. Turn-Key-Tech LLC*, 381 F.3d 1142, 1156, 72 U.S.P.Q.2d 1190, 1200 (Fed. Cir. 2004). Here, the Office issues the rejection without applying the correct legal standard.

“washing the column with 0-10% alcohol (v/v)” in Claim 1

The Office alleges that the Specification does not disclose a limitation in previously presented claim 1, i.e. “washing the column with 0-10% alcohol (v/v).” Applicants traverse.

In this type of purification/enrichment through column chromatography, the meaning of “washing” and “eluting” would be apparent. *Ipsis verbis* support is not the legal requirement. *See e.g., Ex parte Holt*, 19 U.S.P.Q.2d 1211, 1213 (PBAI 1991). Both washing and eluting are steps involved in such column purification/enrichment processes. In pilot experiments to optimize the purification/enrichment conditions, solutions of different compositions are initially used for “eluting.” At this stage, each flow-through is collected and analyzed to determine a range that is most efficient for purification/enrichment.

In at least Examples 3-4 of the Specification, alcohol of 0% (water), 10%, 15%, 20%, and 40% has been used in the experiment to purify SDG. Applicants present findings that (1) SDG is barely washed out by alcohol of 10% or less, and (2) SDG is enriched with alcohol of 15-40%. *See e.g.,* page 17, line 20 to page 28, line 24, and Table 3 of the Specification. Based on at least these descriptions, the ranges of alcohol for washing and eluting are described in the specification and thereby support the claims.

Accordingly, Applicants respectfully request withdrawal of the rejection and allowance of the claims.

“eluting the column with 15 to 40% alcohol (v/v)” in Claim 1

The Office admits that Example 4 of the Specification discloses the elution with 10%, 15%, 20%, and 40% of alcohol (v/v). Nevertheless, the Office alleges that the Specification fails to disclose the 15-40% range, because of the absence of the disclosure of 25% or 30%. Office Action, page 2, last para.

Applicants traverse. To satisfy the written description requirement, neither the statute nor the case law requires the Applicants to disclose each individual concentration within the range of 15-40%. Such a range theoretically contains infinite discrete concentrations, which are impossible to be disclosed individually in any application. Here, the Applicants have presented examples over the claimed range. Additionally, the original claims (*see, e.g.* original claim 4 which describes 30-100% v/v) provide another range. There is sufficient support in the specification for that which is presently claimed.

Accordingly, Applicants respectfully request the withdrawal of the rejection and allowance of the claims.

**“ethanol” in Claim 26**

The Office alleges that the Specification does not disclose washing with ethanol as recited in claim 26. Office Action, page 2, last para.

Applicants traverse. As mention above, the meaning of “washing” or “eluting” should not be interpreted literally in the present application. Applicants direct the Office to page 18, lines 13-14 of the Specification:

SDG was scarcely eluted with *10% ethanol*.

(emphasis added). After determining that ethanol of 10% ethanol or less scarcely eluted SDG, it was determined that 10% or less ethanol was appropriate for washing. In view of the above arguments, Applicants respectfully request the withdrawal of the rejection.

Applicants additionally point out to the Office on page 18, lines 16-19 that the specification further teaches that “elution with 40% ethanol recovered 2.9 g of SDG at a purity of 11.8%. No more SDG was eluted even when the ethanol concentration was further increased.” The specification additionally states “[f]or Example, SDG is preferably eluted at an alcohol concentration of 15% or more” on page 18, lines 21-22. These further descriptions further evince that ranges of alcohol for washing of 0-10% are utilized and for eluting, the ranges of 15% to 40% are utilized.

In view of the above evidence and arguments, the rejection should be withdrawn and the claims allowed.

**5. Rejection of the Claim under 35 U.S.C. § 102(e)**

The Office rejects claims 6 and 28-32 under 35 U.S.C. § 102(e) as allegedly being anticipated by Pihlava et al. (WO 02/062812A1) [hereinafter “Pihlava”].

Without acquiescing as to the merits of the rejection, Applicants have canceled claims 6 and 28-32, thereby mooting the rejection. The rejection can be withdrawn.

**6. Rejection of the Claim under 35 U.S.C. § 103(a)**

**6.1 Rejection over Pihlava**

Claims 1-5 and 25-27 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Pihlava. The Office alleges that Pihlava disclose that:

- 1) an SDG-rich product is extracted from the defatted and crushed flaxseed;
- 2) the defatted flaxseed is extracted with sodium hydroxide-methanol;
- 3) the SDG can be enriched by chromatography, with C18 material packed in a flash chromatography system;
- 4) the SDG can be enriched by washing the loaded column with various alcohol-water mixtures; and
- 5) the SDG can be eluted with 40% methanol.

The Office admits that Pihlava does not disclose:

- 1) the concentration of alcohol used for washing as in claim 1;
- 2) the concentration of alcohol as in claims 4 and 25; and
- 3) the temperature as in claims 27.

The Office alleges that both the temperature and alcohol concentrations are result-effective variables that can be determined by a skilled artisan through routine experimentation. The Office thus concludes that claims 1-5 and 25-27 are obvious over Pihlava.

Applicants traverse. Pihlava discloses isolating and purifying SDG from flaxseed using reverse-phase partition chromatography, which is known in the art as appropriate as a small scale purification technique, not a large scale technique. The current claims utilize adsorption chromatography. Adsorption chromatography methods are appropriate for large-scale industrial purification techniques. Thus the two techniques are based on different principles for different purposes and are not interchangeable for the same problem. For example, adsorption chromatography is less advantageous in terms of purification efficacy than partition chromatography in separating a mixture based on structural differences of substances or in separating a mixture based on structural differences of substances or in separating a mixture based on size of substances. In that regard, Applicants provide an

English translation of the paper entitled "Thin-Layer Chromatography – Basis and Applications."

For the instant claims, the mixture extracted with a basic alcohol from plant material that contains SDG is a mixture comprising different structural substances of difference sizes. Such a mixture would not have a reasonable expectation of purification efficiency using the adsorption chromatography method. However, it was both successful and efficacious and it turned in to a relatively simple process. Identifying a simple, efficacious and successful method of purifying SDG had been sought after.

The reversed-phase partition chromatography employed in Pihlava separates SDG from other substances by differences in partition coefficient of the substances resulting from their differences in partition coefficient of the substances resulting from their differences in hydrophobic interaction with the C18-modified silica particle column. Here, SDG is separated from other substances by the differences in their retention times. To distinguish the differences in the retention times of each substance, the amount of the starting sample loaded to a column must remain small. Thus, Pihlava's teaching cannot be scaled up for large scale purification. Pihlava's method further requires several drying steps. Pihlava further requires repeated column purification steps. *See e.g.*, page 6, lines 10-22 and 24-33 of Pihlava. There is no teaching or suggestion in Pihlava on how these steps could be removed and still efficaciously purify SDG. Additionally, there is no teaching or suggestion to instead use adsorption chromatography *and* remove the other repeated purification steps and drying steps of Pihlava.

The adsorption chromatography of the current claims separate SDG from other substances by trapping it to the HP20 resin. SDG is then desorbed from the resin using alcohol. Adsorption chromatography differs from partition chromatography, as the former includes a desorbing step that removes the trapped SDG from the resin by the alcohol wash. At least one advantage of adsorption chromatography is its ability to process a far larger amount of a starting sample than can be achieved using partition chromatography. This particular advantage lends itself to industrial application. The processing step of the alcohol washes is what allows the claimed method to provide a sufficient amount of SDG in quality and quantity in an efficacious fashion.

In Pihlava, at best only 100 g of defatted powder of flaxseed can be processed. Yet in the instant case at least 1 kg of flax cake is processed. *See e.g.*, Examples 1 and 4 of the specification.

The Office further argues that Pihlava discloses that SDG is eluted with 650 ml of 40% methanol. The Office thus concludes it would have been obvious. This conclusion is unreasonable and unfounded. The solvent used in chromatography depends on the chromatography technique used. The technique used in Pihlava differs from that of the present claims. Yet, this is overlooked by the Office.

As discussed in part above, on page 18, lines 16-19 of the specification, Applicants discuss that elution with 40% ethanol recovered 2.9 g of SDG at a purity of 11.8%. No more SDG was eluted, even when using ethanol concentrations in excess of 40%. Thus, Applicants found that about 40% ethanol is the concentration suited for SDG recovery. The findings are significantly meaningful in recovering SDG by the chromatography technique recited herein.

For at least these reasons, Applicants submit that Pihlava fails to render claims -5 and 25-27 obvious. Accordingly, the rejection should be withdrawn and the claims allowed.

#### 6.2 Rejection over Pihlava in view of Empire

Claims 8-11 and 20-24 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Pihlava in view of Empire et al (U.S. Patent No. 6,391,308) [hereinafter “Empire”].

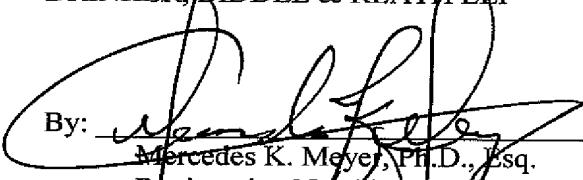
Without acquiescing as to the merits of the rejection, the claims have been cancelled thereby mooted the rejection. The rejection can thus be withdrawn.

**CONCLUSION**

In conclusion, this is believed to be in full response to the outstanding final Office Action. Should any issues remain outstanding or if there are any questions concerning this paper, or the application in general, the Examiner is invited to telephone the undersigned representative at the Examiner's earliest convenience.

Should any outstanding fees be owed or overpayments credited, the Commissioner is invited to charge or credit Deposit Account No. 50-0573. The Office is authorized to charge the Deposit Account for a Notice of Appeal should a Notice of Appeal be necessary to maintain pendency of the application.

Respectfully submitted,  
DRINKER, BIDDLE & REATH LLP

By: 

Mercedes K. Meyer, PH.D., Esq.  
Registration No. 44,939  
1500 K Street, N.W., Suite 1100  
Washington, D.C. 20005-1209  
T: 202-842-8821  
F: 202-842-8465

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Thin-Layer Chromatography  
- Basis and Applications -

Edited by:

Masayuki ISHIKAWA,

Professor at Tokyo Medical and Dental University

Shoji HARA,

Professor at Tokyo University of Pharmacy and Life Sciences

Tsutomu FURUYA,

Professor at Kitasato University

Yasuo NAKAZAWA,

Professor at Tokyo Medical and Dental University

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## 2. Partition chromatography

In a case where a solute is dissolved between two mutually immiscible liquid phases, a concentration equilibrium is established between these phases. In this case, the ratio of the solute concentrations in the respective liquid phases is constant:

$$C_1/C_2 = \alpha: \text{constant}$$

where  $C_1$  and  $C_2$  represent the solute concentrations in the two liquid phases, respectively, and  $\alpha$  is called the partition coefficient.

Partition chromatography is a chromatographic technique for separating a mixture of solutes with different partition coefficients which is carried out by repeatedly partitioning solutes between two liquid phases. In practice of this technique, a carrier having little adsorption capacity (e.g., granular solids or paper filter pieces) is used to hold a highly polar liquid. In this case, the liquid phase held by the carrier serves as the stationary phase. A less polar liquid (mobile phase) saturated with the stationary phase is then passed through interstices in the carrier, whereby the solute will move while a constant concentration equilibrium is maintained between these two phases. The moving velocity may be expressed by Equation (1) shown above, except that  $K$  is replaced by  $\alpha$ . Thus, when the solute is in a mixture form, individual components will form fractionated bands depending on differences in their partition coefficients, thereby allowing qualitative analysis.

Partition chromatography is characterized in that it is also applicable to highly polar water-soluble compounds, and shows a higher resolution than adsorption chromatography when applied to a mixture based on a particular structural difference.

Moreover, theoretical analyses for partition chromatography are in advanced stages, because in partition chromatography a partition coefficient remains constant over a much wider range of concentrations than in adsorption chromatography. However, experimental results do not always accord satisfactorily with theoretical predictions. This is in part because the carrier will produce a slight adsorption effect on solutes although it is originally intended for holding purposes. Moreover, when solutes are acids or bases, a buffer is often used. However, in such a complicated system, it is extremely difficult to theoretically predict an optimum developing solvent and an optimum buffer pH, and hence the only option is to carry out an actual trial by referring to experimental examples, as described above.

In normal partition chromatography, it is common to use a highly polar solvent for the stationary phase. In contrast, a less polar solvent (e.g., paraffin) may be used for the stationary phase, while a highly polar solvent may be used as the mobile phase. Such a technique is especially referred to as "reversed phase chromatography" and is frequently applied in the field of lipids.

#### 4. Selection of chromatography

The above three chromatographic techniques are based on different principles and each has excellent characteristics. It is therefore actually important to recognize the advantages and limitations of each chromatographic technique before addressing the problems of actual separation analysis.

Table 3 summarizes the merits and demerits of separation by each chromatographic technique when applied to compounds with specific structural differences.

This table indicates that adsorption chromatography shows good separation for cis/trans isomers of cyclic compounds, as well as compounds with different numbers of double bonds and with different conjugation states thereof, compounds with different numbers of polar substituents, and compounds with different substituent polarities, whereas it has no effect on compounds with different molecular sizes and optical isomers. On the other hand, in partition chromatography, separation is easier for compounds which have structural differences causing greater changes in the solubility of solutes. For example, partition chromatography is most effective in separating aliphatic analogs with different molecular sizes. Ion exchange chromatography is suitable for ionizable compounds whose ionization degree will vary depending on the hydrogen ion concentration of a solvent, as exemplified by compounds such as amino acids, purines and pteridines. It is also useful in separating macromolecular compounds such as proteins and nucleic acids.

# 薄層クロマトグラフィー

## —基礎と応用—

東京医科歯科大学教授 石川正幸

東京薬科大学教授 原 昭 二 編

北里大学教授 古谷 力

東京医科歯科大学教授 中沢泰男



南山堂

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編者 石原正昭  
谷澤泰正  
中村鈴木二  
著者 石原正昭  
谷澤泰正  
中村鈴木二

発行者

株式会社南山堂

東京都文京区湯島4丁目1-11  
郵便番号113-91  
電話東京(03)811-7241(代)  
振替口座東京6338番

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他の溶媒では円形のスポットになることはしばしば経験されることであり、またある溶媒で一個のスポットであったものが、適切な溶媒では二個のスポットに分離することもまれではなく、展開溶媒がクロマトグラムの良否に与える影響は微妙なものがある。

Trappe<sup>10</sup> は一般的な溶媒をカラムクロマトグラフィーにおける溶出力の順に配列し、溶媒の "eluotropic series" を定めているが、薄層クロマトグラフィーにおいても展開溶媒を選択する基準として価値がある。Strain<sup>11</sup> も同様な順位を定めているが、両者を表 2 に掲げる。Trappe および Strain の順位では、数種の溶媒間に順位の逆転がみられるが、これは、溶媒の溶出力がその極性や電導度などの溶媒の性質のみによって定まるものではなく、溶質によっても微妙に左右されることを示すものである。展開溶媒がおよそどの程度の極性をもつかをあらかじめ試験的に知るためには、Stahl のミクロ円形展開法（後述）が推奨されている。単一の溶媒で目的を達すれば簡単であるが、実際には 2 種あるいは 3 種の溶媒を混合して使用する場合が多く、いかなる溶媒をどのような比率で混合するかは実験者の最も苦心するところである。結局展開溶媒の選択には、類似化合物への応用例を参照し、溶質の化学構造、酸性あるいは塩基性基の有無、溶解度などを考慮して実験的に決定する以外に方法はない。

## 2. 分配クロマトグラフィー

互いに溶解しない二液相間に溶質が溶けているとき、両相間に濃度平衡が成立するが、このときそれぞれの液相中の溶質濃度の比は一定である。すなわち

$$C_1/C_2 = \alpha : \text{一定}$$

ただし  $C_1, C_2$  はそれぞれの液相中の濃度で、 $\alpha$  を分配係数とよぶ。

分配クロマトグラフィーは、分配係数を異にする溶質の混合物を、二液相間に反復して分配して分離するクロマトグラフである。実際にこれを行なうには、ほとんど吸着力のない粒状固体や汎紙片などに、極性の大きい液体を保持させる。この場合保持される液相が固定相になる。つぎに固定相を飽和させた極性の小さい液体（移動相）を保持体の間隙を通って移動させれば、溶質が両相間に濃度平衡を保ちつつ移動することになる。移動速度は (1) 式で  $K$  を  $\alpha$  に読み換えればよい。したがって溶質が混合物のときには、各成分は分配係数の差異に応じて分別帯を形成するから、定性分析が可能になる。

分配クロマトグラフィーの特長は、極性の大きい水溶性化合物にも応用が可能であり、またある種の構造上の差異に基づく混合物には、吸着クロマトグラフィーより優れた分離能を示すことである。分配係数は吸着の場合に較べてはるかに広い濃度範囲で一定となるので、理論的解析も進んだ状態にあるけれども、実験結果と理論とが満足すべき一致を示

しているとは必ずしもいえない。本来は 固定相保持を目的とする担体が若干の吸着作用を溶質に及ぼすこともその原因であろうし、また 溶質が酸あるいは塩基の場合緩衝液を使用することが多いが、このような複雑な系になると、最も効果的な展開溶媒、緩衝液の pH 値を理論的に予測することは極めて困難であって、前述のように実験例を参照にして実際に試みる以外に方法はない。

一般的分配クロマトグラフィーでは、固定相に極性の大きい溶媒を使用するのが普通であるが、逆に固定相にパラフィンのような極性の小さい溶媒を使用し、極性の大きい方を移動相とすることがある。この方法は特に“逆相クロマトグラフィー *reversed phase chromatography*”とよばれ、脂質の分野ではしばしば応用されている。

### 3. イオン交換クロマトグラフィー

イオン交換クロマトグラフィーは主としてカラム法でアミノ酸、無機イオン類の分離に利用されている。薄層クロマトグラフィーでは、イオン交換樹脂を吸着剤に応用した例は多くはないが、Randerath は核酸および核酸誘導体の分離にイオン交換処理を施したセルロース粉末の薄層で優れた分離結果を得ている。イオン交換手法の詳細については、その専門書<sup>\*</sup>を参考にされたい。

### 4. クロマトグラフィーの選択

上述の三種類のクロマトグラフィーは、原理を異にし、それぞれ優れた特長をもっているから、実際の分離分析の問題にあたって、各々のクロマトグラフィーの長所と限界を理解しておくことは、実際問題として大切なことである。

表3<sup>14</sup>は特定の構造上の差異をもった化合物に対して、それぞれのクロマトグラフィーによる分離の優劣を示したものである。

これによると吸着クロマトグラフィーは、環状化合物のシス・トランス異性体、二重結合数を異にするもの、およびその性質が異なっているもの、有機性脂族基を異にするもの、置換基の極性に差があるものなどに良好な分離を示すが、分子の大きさの異なるもの、光学異性体には無力であることがわかる。一方分配クロマトグラフィーでは、溶質の溶解度により多くの変化を与える構造上の差のあるものほど分離は容易となる。たとえば分子の大きさを異にする脂肪酸同族体の分離には分配クロマトグラフィーは最も

\* C. Calmon, T. Grossman : *Ion Exchangers in Organic and Biochemistry* Interscience publishers, Inc., New York, N. Y.  
垣花、成田：イオン交換 広川書店。

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優れている。イオン交換クロマトグラフィーではイオン化の可能な化合物で、しかも溶媒の水素イオン濃度によってそのイオン化の程度が変化するもの、たとえばアミノ酸、ブリンおよびブテリシンなどの化合物には適切であり、また蛋白質、核酸などの高分子化合物に対する分離にも有用である。

表 3.

構造上の差	吸着クロマト グラフィー	分配クロマト グラフィー	イオン交換クロ マトグラフィー
分子の大きさ	+-+(+)	+-++	+-++
構造異性体			
鎖状ならびに環状化合物	+-+(+)	+	-
分枝した鎖状化合物	+-+(+)	(+)	(+)
二重結合の位置	+-+(+)	-	-
立体異性体			
二重結合におけるシス・トランス	+-+(+)	+	+
環状化合物のシス・トランス	+-+(+)	+	+
光学異性体	(+)	(+)	+-+(+)
二重結合の数	++	+	-
二重結合の共役	++-+	+	+
無極性置換基の数	+-+-(+)	+-+(+)	+-+(+)
有極性置換基の数	++	++	++
置換基の属性	++	++	++

++: 非常によい, +: よい, (+): よくない, -: 研究されていない

## 文 献

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